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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT: Vernet, et al.
SERIAL NUMBER: 09/825,751 EXAMINER: Misook Yu
FILING DATE: April 3, 2001 ART UNIT: 1642
FOR: Novel Proteins and Nucleic Acids Encoding Same

Commissioner for Patents
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. § 1.132

I, Meera Patturajan hereby declare and state as follows:

1. I am employed by CuraGen, Inc., the assignee of this application. My title is research scientist. I received a Ph.D. in Microbiology and Cell biology from the Indian Institute of Science, India, where I studied the purification and characterization of RNA Polymerase II from Candida Utilis. I was a postdoctoral fellow at the Johns Hopkins University from 1995-2000, where I was involved in understanding the role of proteins involved in oncogenesis and also the role of RNA polymerase II in transcription and mRNA splicing.

2. I have read, and am familiar with, the contents of the United States patent application entitled "Novel Proteins and Nucleic Acids Encoding Same", serial number 09/825,751, which was filed April 3, 2001. I understand that the pending claims are directed to an isolated polypeptide comprising SEQ ID NO:20.

3. I am aware that the Examiner has issued an Office Action. In particular, I understand that the Examiner has rejected the pending claims under 35 U.S.C. § 101 contending that the pending claims are not supported by either a specific and substantial asserted utility or a well-established utility.

4. I make this declaration to rebut the Examiner's assertion, with which I do not agree. It is my opinion that the claimed compositions have a specific and substantial utility for at least the following reasons.

5. I have performed, or have had performed under my supervision, studies evaluating the quantitative expression and sequence homology of the nucleic acid encoding the claimed polypeptide of SEQ ID NO:20 in tissue culture cells and in isolated normal and pathological human tissue.

6. In a first study, experiments using probe/primer sets gave results, shown in Table AB in the Appendix, that show that expression of the gene is highest in an ovarian cancer cell line (CT=27.5), high to moderate expression of this gene is also seen in cell lines derived from ovarian, lung, renal and brain (gliomas and astrocytomas) cancers which is in concordance with the data provided in the original specification (Table 22, pg. 124). Therefore, therapeutic modulation of this gene or its protein product may be useful in the treatment of these cancers.

7. In a second study, experiments using probe/primer sets gave results, shown in Table AC in the Appendix, showing that significant expression of this gene is seen in cell lines derived from glioma, blastoglioma, lung and bone cancers, although highest expression of this gene is seen in small cell lung cancer cell line.

8. The results of these studies, in my opinion, demonstrate consistent increased expression in SF-268 (astrocytoma and glioblastoma) in both Panels v1.7 and v3.2, indicating that nucleic acid encoding the claimed polypeptide of SEQ ID NO:20 can be used for diagnosis and treatment of cancers involving the Central Nervous System. Thus, I believe that the Examiner should withdraw the rejection and allow the pending claims.

9. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001, Title 18, United States Code, and that willful false statements may jeopardize the validity of this application and any patent issuing therefrom.

Meera Patturajan

Meera Patturajan, Ph. D

Signed at Branford, CT this 3rd day of March '03

Appendix

Quantitative expression analysis of clones in various cells and tissues

The quantitative expression of various clones was assessed using microtiter plates containing RNA samples from a variety of normal and pathology-derived cells, cell lines and tissues using real time quantitative PCR (RTQ PCR). RTQ PCR was performed on an Applied Biosystems ABI PRISM® 7700 or an ABI PRISM® 7900 HT Sequence Detection System. Various collections of samples are assembled on the plates, and referred to as Panel 1 (containing normal tissues and cancer cell lines), Panel 2 (containing samples derived from tissues from normal and cancer sources), Panel 3 (containing cancer cell lines), Panel 4 (containing cells and cell lines from normal tissues and cells related to inflammatory conditions), Panel 5D/5I (containing human tissues and cell lines with an emphasis on metabolic diseases), AI_comprehensive_panel (containing normal tissue and samples from autoinflammatory diseases), Panel CNSD.01 (containing samples from normal and diseased brains) and CNS_neurodegeneration_panel (containing samples from normal and Alzheimer's diseased brains).

RNA integrity from all samples is controlled for quality by visual assessment of agarose gel electropherograms using 28S and 18S ribosomal RNA staining intensity ratio as a guide (2:1 to 2.5:1 28s:18s) and the absence of low molecular weight RNAs that would be indicative of degradation products. Samples are controlled against genomic DNA contamination by RTQ PCR reactions run in the absence of reverse transcriptase using probe and primer sets designed to amplify across the span of a single exon.

First, the RNA samples were normalized to reference nucleic acids such as constitutively expressed genes (for example, β-actin and GAPDH). Normalized RNA (5 μl) was converted to cDNA and analyzed by RTQ-PCR using One Step RT-PCR Master Mix Reagents (Applied Biosystems; Catalog No. 4309169) and gene-specific primers according to the manufacturer's instructions.

In other cases, non-normalized RNA samples were converted to single strand cDNA (sscDNA) using Superscript II (Invitrogen Corporation; Catalog No. 18064-147) and random hexamers according to the manufacturer's instructions. Reactions containing up to 10 μg of total RNA were performed in a volume of 20 μl and incubated for 60 minutes at 42°C. This reaction can be scaled up to 50 μg of total RNA in a final volume of 100 μl. sscDNA samples are then

normalized to reference nucleic acids as described previously, using 1X TaqMan® Universal Master mix (Applied Biosystems; catalog No. 4324020), following the manufacturer's instructions.

Probes and primers were designed for each assay according to Applied Biosystems Primer Express Software package (version I for Apple Computer's Macintosh Power PC) or a similar algorithm using the target sequence as input. Default settings were used for reaction conditions and the following parameters were set before selecting primers: primer concentration = 250 nM, primer melting temperature (Tm) range = 58°-60°C, primer optimal Tm = 59°C, maximum primer difference = 2°C, probe does not have 5'G, probe Tm must be 10°C greater than primer Tm, amplicon size 75bp to 100bp. The probes and primers selected (see below) were synthesized by Synthegen (Houston, TX, USA). Probes were double purified by HPLC to remove uncoupled dye and evaluated by mass spectroscopy to verify coupling of reporter and quencher dyes to the 5' and 3' ends of the probe, respectively. Their final concentrations were: forward and reverse primers, 900nM each, and probe, 200nM.

PCR conditions: When working with RNA samples, normalized RNA from each tissue and each cell line was spotted in each well of either a 96 well or a 384-well PCR plate (Applied Biosystems). PCR cocktails included either a single gene specific probe and primers set, or two multiplexed probe and primers sets (a set specific for the target clone and another gene-specific set multiplexed with the target probe). PCR reactions were set up using TaqMan® One-Step RT-PCR Master Mix (Applied Biosystems, Catalog No. 4313803) following manufacturer's instructions. Reverse transcription was performed at 48°C for 30 minutes followed by amplification/PCR cycles as follows: 95°C 10 min, then 40 cycles of 95°C for 15 seconds, 60°C for 1 minute. Results were recorded as CT values (cycle at which a given sample crosses a threshold level of fluorescence) using a log scale, with the difference in RNA concentration between a given sample and the sample with the lowest CT value being represented as 2 to the power of delta CT. The percent relative expression is then obtained by taking the reciprocal of this RNA difference and multiplying by 100.

When working with sscDNA samples, normalized sscDNA was used as described previously for RNA samples. PCR reactions containing one or two sets of probe and primers were set up as described previously, using 1X TaqMan® Universal Master mix (Applied Biosystems; catalog No. 4324020), following the manufacturer's instructions. PCR amplification

was performed as follows: 95°C 10 min, then 40 cycles of 95°C for 15 seconds, 60°C for 1 minute. Results were analyzed and processed as described previously.

General screening panel v1.4, v1.5, v1.6 and 1.7

The plates for Panels 1.4, 1.5, 1.6 and 1.7 include 2 control wells (genomic DNA control and chemistry control) and 88 to 94 wells containing cDNA from various samples. The samples in Panels 1.4, 1.5, 1.6 and 1.7 are broken into 2 classes: samples derived from cultured cell lines and samples derived from primary normal tissues. The cell lines are derived from cancers of the following types: lung cancer, breast cancer, melanoma, colon cancer, prostate cancer, CNS cancer, squamous cell carcinoma, ovarian cancer, liver cancer, renal cancer, gastric cancer and pancreatic cancer. Cell lines used in Panels 1.4, 1.5, 1.6 and 1.7 are widely available through the American Type Culture Collection (ATCC), a repository for cultured cell lines, and were cultured using the conditions recommended by the ATCC. The normal tissues found on Panels 1.4, 1.5, 1.6 and 1.7 are comprised of pools of samples derived from all major organ systems from 2 to 5 different adult individuals or fetuses. These samples are derived from the following organs: adult skeletal muscle, fetal skeletal muscle, adult heart, fetal heart, adult kidney, fetal kidney, adult liver, fetal liver, adult lung, fetal lung, various regions of the brain, the spleen, bone marrow, lymph node, pancreas, salivary gland, pituitary gland, adrenal gland, spinal cord, thymus, stomach, small intestine, colon, bladder, trachea, breast, ovary, uterus, placenta, prostate, testis and adipose. Abbreviations are as described below:

ca. = carcinoma,
* = established from metastasis,
met = metastasis,
s cell var = small cell variant,
non-s = non-sm = non-small,
squam = squamous,
pl. eff = pl effusion = pleural effusion,
glio = glioma,
astro = astrocytoma, and
neuro = neuroblastoma.

Panel 3D and 3.1 and 3.2

The plates of Panel 3D, 3.1, and 3.2 are comprised of 94 cDNA samples and two control samples. Specifically, 92 of these samples are derived from cultured human cancer cell lines, 2 samples of human primary cerebellar tissue and 2 controls. The human cell lines are generally obtained from ATCC (American Type Culture Collection), NCI or the German tumor cell bank and fall into the following tissue groups: Squamous cell carcinoma of the tongue, breast cancer, prostate cancer, melanoma, epidermoid carcinoma, sarcomas, bladder carcinomas, pancreatic cancers, kidney cancers, leukemias/lymphomas, ovarian/uterine/cervical, gastric, colon, lung and CNS cancer cell lines. In addition, there are two independent samples of cerebellum. These cells are all cultured under standard recommended conditions and RNA extracted using the standard procedures. The cell lines in panels 3D, 3.1, 3.2, 1, 1.1., 1.2, 1.3D, 1.4, 1.5, and 1.6 are some of the most common cell lines used in the scientific literature.

A. G55707_A: Growth/differentiation factor 6 precursor.

Expression of gene G55707_A was assessed using the primer-probe sets Ag7087, described in Tables AA. Results of the RTQ-PCR runs are shown in Tables AB and AC.

Table AA. Probe Name Ag7087

Primers	Sequences	Length	Start Position
Forward	5'-agctttgtagacaggggactagac-3'	24	500
Probe	TET-5'-atctctcgcacactcctctccggag-3'-TAMRA	25	525
Reverse	5'-ggacacatcaaacaaatacttctgt-3'	25	550

Table AB. General_screening_panel_v1.7

Column A - Rel. Exp.(%) Ag7087, Run 318036513			
Tissue Name	A	Tissue Name	A
Adipose	9.3	Gastric ca. (liver met.) NCI-N87	0.0
HUVEC	56.3	Stomach	0.1
Melanoma* Hs688(A).T	0.0	Colon ca. SW-948	0.0
Melanoma* Hs688(B).T	0.0	Colon ca. SW480	0.0
Melanoma (met) SK-MEL-5	0.0	Colon ca. (SW480 met) SW620	0.0
Testis	0.5	Colon ca. HT29	0.0
Prostate ca. (bone met) PC-3	2.1	Colon ca. HCT-116	0.0
Prostate ca. DU145	0.0	Colon cancer tissue	0.2
Prostate pool	0.7	Colon ca. SW1116	0.0

Uterus pool	0.5	Colon ca. Colo-205	0.0
Ovarian ca. OVCAR-3	0.1	Colon ca. SW-48	0.0
Ovarian ca. (ascites) SK-OV-3	0.0	Colon	2.1
Ovarian ca. OVCAR-4	0.7	Small Intestine	1.1
Ovarian ca. OVCAR-5	2.4	Fetal Heart	3.0
Ovarian ca. IGROV-1	0.0	Heart	1.0
Ovarian ca. OVCAR-8	**100.0**	Lymph Node pool 1	1.2
Ovary	1.9	Lymph Node pool 2	8.5
Breast ca. MCF-7	0.0	Fetal Skeletal Muscle	
Breast ca. MDA-MB-231	0.0	Skeletal Muscle pool	0.1
Breast ca. BT-549	0.6	Skeletal Muscle	0.4
Breast ca. T47D	0.0	Spleen	0.3
Breast pool	4.7	Thymus	0.0
Trachea	2.6	CNS cancer (glio/astro) SF-268	27.5
Lung	2.6	CNS cancer (glio/astro) T98G	0.2
Fetal Lung	4.2	CNS cancer (neuro;met) SK-N-AS	0.0
Lung ca. NCI-N417	0.0	CNS cancer (astro) SF-539	4.6
Lung ca. LX-1	0.0	CNS cancer (astro) SNB-75	1.0
Lung ca. NCI-H146	0.0	CNS cancer (glio) SNB-19	3.4
Lung ca. SHP-77	0.0	CNS cancer (glio) SF-295	0.0
Lung ca. NCI-H23	1.1	Brain (Amygdala)	1.8
Lung ca. NCI-H460	0.0	Brain (Cerebellum)	0.5
Lung ca. HOP-62	0.4	Brain (Fetal)	8.3
Lung ca. NCI-H522	2.5	Brain (Hippocampus)	1.4
Lung ca. DMS-114	73.2	Cerebral Cortex pool	1.6
Liver	1.4	Brain (Substantia nigra)	0.7
Fetal Liver	3.7	Brain (Thalamus)	2.9
Kidney pool	1.4	Brain (Whole)	5.7
Fetal Kidney	3.0	Spinal Cord	0.7
Renal ca. 786-0	9.1	Adrenal Gland	1.6
Renal ca. A498	0.0	Pituitary Gland	0.7
Renal ca. ACHN	2.5	Salivary Gland	0.0
Renal ca. UO-31	0.0	Thyroid	4.0
Renal ca. TK-10	0.0	Pancreatic ca. PANC-1	0.0
Bladder	10.1	Pancreas pool	1.3

Table AC. Oncology_cell_line_screening_panel_v3.2

Column A - Rel. Exp.(%) Ag7087, Run 323342064			
Tissue Name	A	Tissu Name	A
94905_Daoy_Medulloblastoma/Cerebellum_sscDNA	0.0	94954_Ca Ski_Cervical epidermoid carcinoma (metastasis)_sscDNA	0.0
94906_TE671_Medulloblastom/Cerebellum_sscDNA	0.0	94955_ES-2_Ovarian clear cell carcinoma_sscDNA	0.2
94907_D283 Med_Medulloblastoma/Cerebellum_sscDNA	0.0	94957_Ramos/6h stim_ Stimulated with PMA/ionomycin 6h_sscDNA	0.0
94908_PFSK-1_Primitive Neuroectodermal/Cerebellum_sscDNA	0.0	94958_Ramos/14h stim_ Stimulated with PMA/ionomycin 14h_sscDNA	0.0
94909_XF-498_CNS_sscDNA	10.2	94962_MEG-01_Chronic myelogenous leukemia (megokaryoblast)_sscDNA	0.6
94910_SNB-78_CNS/glioma_sscDNA	27.2	94963_Raji_Burkitt's lymphoma_sscDNA	0.0
94911_SF-268_CNS/glioblastoma_sscDNA	38.4	94964_Daudi_Burkitt's lymphoma_sscDNA	0.0
94912_T98G_Glioblastoma_sscDNA	0.0	94965_U266_B-cell plasmacytoma/myeloma_sscDNA	0.0
96776_SK-N-SH_Neuroblastoma (metastasis)_sscDNA	3.3	94968_CA46_Burkitt's lymphoma_sscDNA	0.0
94913_SF-295_CNS/glioblastoma_sscDNA	0.6	94970_RL_non-Hodgkin's B-cell lymphoma_sscDNA	0.0
132565_NT2 pool_sscDNA	3.2	94972_JM1_pre-B-cell lymphoma/leukemia_sscDNA	0.0
94914_Cerebellum_sscDNA	0.0	94973_Jurkat_T cell leukemia_sscDNA	0.3
96777_Cerebellum_sscDNA	0.3	94974_TF-1_Erythroleukemia_sscDNA	0.0
94916_NCI-H292_Mucoepidermoid lung carcinoma_sscDNA	0.0	94975_HUT 78_T-cell lymphoma_sscDNA	0.0
94917_DMS-114_Small cell lung cancer_sscDNA	**100.0**	94977_U937_Histiocytic lymphoma_sscDNA	0.0
94918_DMS-79_Small cell lung cancer/neuroendocrine_sscDNA	1.0	94980_KU-812_Myelogenous leukemia_sscDNA	0.0
94919_NCI-H146_Small cell lung cancer/neuroendocrine_sscDNA	0.0	94981_769-P_Clear cell renal carcinoma_sscDNA	0.0
94920_NCI-H526_Small cell lung cancer/neuroendocrine_sscDNA	0.0	94983_Caki-2_Clear cell renal carcinoma_sscDNA	0.0
94921_NCI-N417_Small cell lung cancer/neuroendocrine_sscDNA	0.0	94984_SW 839_Clear cell renal carcinoma_sscDNA	0.0

94923_NCI-H82_Small cell lung cancer/neuroendocrine_sscDNA	0.0	94986_G401_Wilms' tumor_sscDNA	0.2
94924_NCI-H157_Squamous cell lung cancer (metastasis)_sscDNA	2.8	126768_293 cells_sscDNA	0.0
94925_NCI-H1155_Large cell lung cancer/neuroendocrine_sscDNA	1.3	94987_Hs766T_Pancreatic carcinoma (LN metastasis)_sscDNA	0.0
94926_NCI-H1299_Large cell lung cancer/neuroendocrine_sscDNA	18.9	94988_CAPAN-1_Pancreatic adenocarcinoma (liver metastasis)_sscDNA	0.0
94927_NCI-H727_Lung carcinoid_sscDNA	0.4	94989_SU86.86_Pancreatic carcinoma (liver metastasis)_sscDNA	0.0
94928_NCI-UMC-11_Lung carcinoid_sscDNA	0.0	94990_BxPC-3_Pancreatic adenocarcinoma_sscDNA	0.0
94929_LX-1_Small cell lung cancer_sscDNA	0.0	94991_HPAC_Pancreatic adenocarcinoma_sscDNA	0.0
94930_Colo-205_Colon cancer_sscDNA	0.0	94992_MIA PaCa-2_Pancreatic carcinoma_sscDNA	0.0
94931_KM12_Colon cancer_sscDNA	0.0	94993_CFPAC-1_Pancreatic ductal adenocarcinoma_sscDNA	0.0
94932_KM20L2_Colon cancer_sscDNA	0.0	94994_PANC-1_Pancreatic epithelioid ductal carcinoma_sscDNA	0.0
94933_NCI-H716_Colon cancer_sscDNA	0.0	94996_T24_Bladder carcinma (transitional cell)_sscDNA	0.0
94935_SW-48_Colon adenocarcinoma_sscDNA	0.0	94997_5637_Bladder carcinoma_sscDNA	0.0
94936_SW1116_Colon adenocarcinoma_sscDNA	0.0	94998_HT-1197_Bladder carcinoma_sscDNA	0.0
94937_LS 174T_Colon adenocarcinoma_sscDNA	0.0	94999_UM-UC-3_Bladder carcinma (transitional cell)_sscDNA	1.1
94938_SW-948_Colon adenocarcinoma_sscDNA	0.0	95000_A204_Rhabdomyosarcoma_sscDNA	0.0
94939_SW-480_Colon adenocarcinoma_sscDNA	0.0	95001_HT-1080_Fibrosarcoma_sscDNA	2.8
94940_NCI-SNU-5_Gastric carcinoma_sscDNA	0.0	95002_MG-63_Osteosarcoma (bone)_sscDNA	77.4
112197_KATO III_Stomach_sscDNA	0.0	95003_SK-LMS-1_Leiomyosarcoma (vulva)_sscDNA	0.0
94943_NCI-SNU-16_Gastric carcinoma_sscDNA	0.0	95004_SJRH30_Rhabdomyosarcoma (met to bone marrow)_sscDNA	0.0
94944_NCI-SNU-1_Gastric carcinoma_sscDNA	0.0	95005_A431_Epidermoid carcinoma_sscDNA	0.0
94946_RF-1_Gastric adenocarcinoma_sscDNA	0.0	95007_WM266-4_Melanoma_sscDNA	0.0

94947_RF-48_Gastric adenocarcinoma_sscDNA	0.0	112195_DU 145_Prostate_sscDNA	0.0
96778_MKN-45_Gastric carcinoma_sscDNA	0.0	95012_MDA-MB-468_Breast adenocarcinoma_sscDNA	0.0
94949_NCI-N87_Gastric carcinoma_sscDNA	0.0	112196_SSC-4_Tongue_sscDNA	0.0
94951_OVCAR-5_Ovarian carcinoma_sscDNA	0.0	112194_SSC-9_Tongue_sscDNA	0.0
94952_RL95-2_Uterinc carcinoma_sscDNA	2.7	112191_SSC-15_Tongue_sscDNA	0.1
94953_HelaS3_Cervical adenocarcinoma_sscDNA	0.0	95017_CAL 27_Squamous cell carcinoma of tongue_sscDNA	0.0